23136

IN THE U.S. PATENT AND TRADEMARK OFFICE

Inventor Juergen DOLDERER

Patent App. 10/516,521

Filed 2 May 2005 Conf. No. 4497

For DIAGNOSTIC AGENT AND METHOD FOR DETECTION OF CARCINOMA, AND MEANS FOR TREATMENT THEREOF

Art Unit 1612 Examiner Sutton, D

Hon. Commissioner of Patents
Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR 1 132

I, Dr. Juergen H. Dolderer, MD, a citizen of the Federal Republic of Germany, residing at 13 Karl-Adam-Strasse, 72076 Tuebingen, Federal Republic of Germany, declare as follows:

THAT I am a medical doctor with the degree of doctor of medicine awarded to me from the Johann Wolfgang Goethe-University of Frankfurt am Main, Federal Re public of Germany;

THAT I am a licensed medical surgeon in the Federal Republic of Germany;

THAT I have practiced oncology, including cancer surgery, for a number of years in the Federal Republic of Germany and at the University of Melbourne at the St. Vincent Hospital in Melbourne, Australia;

THAT my full curriculum vitae may be attached hereto;

THAT I am the Applicant in US Patent Application Serial No. 10/516,521 filed 2 May 2005 and directed to DIAGNOSTIC AGENT AND METHOD FOR THE DETECTION OF CARCINOMA, AND MEANS FOR THE TREATMENT THEREOF;

THAT based upon my knowledge of and experience in the field of oncology, I can state that all tumors in general and all adenocarcinomas in particular, do not necessarily express the hERG potassium ion channel, and in fact the majority of tumors do not express the hERG potassium ion channel;

THAT in support of my statement I wish to provide the following explanation and support in the medical literature:

If Bianchi et al in "herg Encodes a K+ Current Highly Conserved in Tumors of Different Histogenesis: A selective Advantage for Cancer Cells" in Cancer Research 58, 815 to 822, February 15, 1998, cited by the Examiner in the prosecution of the present application, suggested that all immortalized cell lines express KCNH2 (official name for hERG1) , and which in turn would suggest that all cancerous (immortalized) cells also express KCNH2, then all cell lines would need to express KCNH2 in order to confer the transformed phenotype to the cell lines. Secondly, the cell lines would need to be a valid model for tissue in or ex vivo;

The paper of Lastraioli et al."herg1 gene and HERG1 Protein Are Overexpressed in Colorectal Cancers and Regulate Cell Invasion of Tumor Cells"; Cancer Research 64. 606 to 611, January 15, 2004, which I have designated as "Attachment 1" Figure 1 demonstrates that HEK 293 cells express little or no KCNH2 until the gene is transfected into the cells. Additional results in the same figure show that the level of KCNH2 expression in different cell lines of colorectal origin vary a great deal, clearly demonstrating that a high level of expression is not required for the transformed phenotype in vitro.

In Figure 4.A in the paper of Zhao et al. "HERG K+ Chanel Blockade by the Novel Antiviral Drug Sophocarpine"; Biol. Pharm.Bull. 31(4) 627 to 632 (2008), which I have designated as "Attachment 2", there is confirmation of the very low level of expression of KCNH2 in untransfected. HEK293 cells;

In Kupershmidt et. al. I_{Kr} drug response is modulated by KCR1 in transfected cardiac and noncardiac cell lines", The FASEB Journal, Express Article, 10.1096/fj.02-1057fje, published on line October 2, 2003, which I have designated as "Attachment 3", the authors were not able to detect KCNH2 in tsA201 cells prior to transfection at all (see Figure 1.11). This proves that KCNH2 is not a universal property of all cell lines, as perhaps speculated by Bianchi et al;

I now offer the following additional explanation: the data extracted from the publications demonstrate that the expression pattern of hERG varies considerably in different cell lines, even from colorectal origin. In some cell lines, such as HEK 293, hERG is expressed at levels so low that the protein cannot be detected by Western blot until hERG is transfected into the cells to increase the expression level. This was

not a point of import in the papers, it is simply that the papers contain data relative to this point.

I believe that what is key is that Bianchi et al. presented results from a mere six cell lines and created the impression that hERG is expressed in all adenocarcinoma cell lines, if not in all cancer cell lines, and that the hERG is essential for all oncogenic transformations. I strongly disagree that it is correct to use this highly limited set of data set from Bianchi et al, and to rely on Bianchi et al's, admitted mere speculation to support the conclusion that my finding hERG expressed selectively in cancerous colorectal tissue was obvious. The data presented in Lastraioli et al, Zhao et al, and Kupershmidt et al show that hERG is not expressed in all cancer cell lines, and is not expressed equally in all cell lines, even of colorectal origin. Thus any conclusion, that consistent expression in cell lines makes expression in all tissues, including colorectal carcinomas, obvious, is revealed to be based on a very limited set of data. When the additional data are considered, any such conclusion of obviousness is clearly not consistent with the data as a whole and thus expression of hERG potassium ion channels in colorectal cancer tissues cannot be considered obvious.

My results in the present application demonstrate that KCNH2 is expressed by colorectal carcinomas so after taking the results of my present invention into consideration it is hardly surprising that cell lines derived from these tissues express KCNH2 at some level. But the variability observed by Lastraioli et al. suggests it is not essential for growth in tissue culture.

The results from Patt et al, "Expression of ether à go-go potassium channels in human gliomas", Neuroscience Letters, 368, (2004), pp 249 to 253, especially in figure 1, p. 251, which I have designated as "Attachment 4", show the same pattern in glioma cells, with some cells expressing high levels and others only weak levels of KCNH2 (hERG1). Their observations support Bianchi et al's speculation concerning a role for hERG in astrocytomas (low grade tumor) , but not for (oligodendrogliomas) high grade tumors. Patt et al conclude "hERG expression in gliomas thus seems to be different from that in non-neural tumors". The existence of this difference was impossible to predict a priori. For the same reason, it was impossible to know a priori if hERG expression was diagnostic for the presence of tumors in colorectal tissue, as I have first demonstrated according to my present invention.

Cell lines have been used for years as the work horse of molecular biology, due to their ease of handling and widespread availability. Nevertheless, in recent years efforts have intensified to come up with alternative systems based on primary or stem cells to compensate for the inherent weakness of cell lines as model systems for in vivo processes. The transformed phenotype of cell lines means that by definition, some key regulatory processes are changed relative to untransformed cells.

In the context of the Bianchi et al paper, the finding that cell lines of various origins express KCNH2 could have reflected a selection for KCNH2 in vitro. Most fresh cell material taken from a tumor will grow for a time in culture, but then goes through a crisis, during which most of the cells die. The remaining cells are clearly selected for growth under the conditions of tissue culture. Thus Bianchi's speculation comparing their very limited in vitro results with cell lines for tumors other than colorectal tumor cells, cannot be viewed as apparent or even particularly likely with respect to my research on colorectal tumors in tissue. Thus a critical component of my research nowhere to be found in Bianchi et al, was my systematic investigation of primary tissue taken from patients with colorectal cancer that demonstrated the consistent expression of KCNH2 in cancerous, but not in normal tissue of adults.

THAT I am aware of no data inconsistent with the data presented above, or which would lead one to a contrary conclusion; and

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 USC 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

June 23, 2008
Date

Dr. Juergen H. Dolderer, MD

Enc: 4 PUBLICATIONS

CURRICULUM VITAE

PERSONAL DETAILS

Name	Juergen H. DOLDERER
Current Address	13 Karl-Adam-Strasse 72076 Tuebingen Germany Tel: +49-177-3060185 Fax: +49-7071-9692907 Email: drdolderer@hotmail.com
Marital Status	Married – Annette Dolderer Son Benedikt
Date of birth	July 7, 1968
Nationality	German
Academic Title	MD (Medical Doctor)

CURRENT POSITION

Consultant Surgeon
at the Medical Center of the University of Tuebingen,
BG-Trauma Center, Department of Reconstructive Surgery,
Tuebingen, Germany

QUALIFICATIONS

12/2006	Consultant Surgeon
06/2001	Specialisation in Emergency Medicine
06/2000	Specialisation in Surgical Oncology
06/1999	Specialisation in Surgical Ultrasound
10/1998	Degree Medical Doctor (MD Thesis)
11/1997	Graduation from Medical School
09/1990	Medical-Technical-Laboratory-Assistant

UNIVERSITY EDUCATION

1988-1990	Medical-Technical-Laboratory-Assistant Education in Stuttgart-Germany
1990-1997	Study of Medicine: University of Frankfurt-Germany
	University of Zürich-Switzerland, Dept. of Anaesthesia and Intensive Care (Th. Pasch)
	University of Cape Town-South Africa Dept. of Internal Medicine (S. Joubert)
	University of California in San Francisco-USA Dept. of Trauma Surgery (W. Schecter) Dept. of Reconstructive Surgery (S. Mathes)

HOSPITAL APPOINTMENTS

1997-1998	Surgical Resident in Abdominal and Trauma Surgery, Hl. Geist-Hospital Bensheim, Germany (Dr. R. Blagojevic, Dr. S. Forell)
1998	Surgical Resident in the Dept. of Surgical Intensive Care Unit, Nordwest-Hospital, Frankfurt-Germany (Dr. B. Böhm)
1998-2001	Dept. of Surgery, Center for Oncologic Surgery, Nordwest-Hospital, Teaching Hospital of the Goethe-University, Frankfurt, Germany (Prof. H. Bockhorn)
2001-2004	Clinical and Research Fellowship in Microsurgery at the Bernard O'Brien Institute of Microsurgery and Dept. of Reconstructive Surgery, University of Melbourne, St. Vincent's Hospital, Melbourne, Australia (Prof. W. Morrison)

2004-2005	Advanced Trainee in the Dept. of Reconstructive and Hand Surgery, Burn-Center, BG-Trauma-Hospital Ludwigshafen, University of Heidelberg, Germany (Prof. G. Germann)
2006	Advanced Trainee in the Dept. of General, Trauma and Colorectal Surgery, Frankenthal Hospital, Frankenthal, Germany (Prof. J. Reiter)
2007	Consultant Surgeon in the Dept. of Reconstructive, Oncologic and Breast Surgery, Medical Center Mittelbaden, Rastatt, Germany (Dr. Dr. R. Herr)
2007-today	Consultant Surgeon in the Department of Reconstructive Surgery, Medical Center of the University of Tuebingen, BG-Trauma Center, Tuebingen, Germany (Prof. H.-E. Schaller)

CLINICAL HUMANITARIAN SUPPORT and SURGERY OPERATION MISSION

1996	Kilimanjaro Christian Medical Center, Moshi, Tanzania
2007	Agona Swedru, Central District, Ghana

TRAINING COURSES

1992	Advanced Cardiac Life Support Course, 97th General Hospital, Frankfurt-Germany (Dr. A. Kellogg)
1994	Course in Microsurgical Anastomosis, Harry Buncke Microsurgical-Institute, San Francisco-USA (Dr. Harry Buncke)

1994-1995	Assistant at the Dr. Senckenberg-Institute for Anatomy, Goethe-University, Frankfurt-Germany (Prof. Korf) Teaching of Medical Students
1998, 1999	Course in Basic and Advanced Surgical Ultrasonography, Hamburg-Germany
1998	Workshop of Surgical Trauma Management, Frankfurt-Germany
1998	Postgraduate Course in Advanced Onco-Surgery and Course in Surgical Research, Bern-Switzerland (Prof. Büchler)
1999	Course Emergency Medicine and Surgery, Garmisch-Partenkirchen-Germany
1999	Course Experimental Microsurgery, Ethicon Inc., Hamburg-Germany
1999	Course Laparoscopic Surgery, Ethicon Inc., Hamburg-Germany
2000	Course Pediatric Plastic & Maxillofacial Surgery, Royal Australasian College of Plastic Surgeons, University of Melbourne-Australia (Prof. A. Holmes)
2000	Workshop in Experimental Methods in Surgical Research, Malmö-Sweden (Prof. A. Montgomery)
2001	Course Laparoscopic Surgery, Städt. Klinikum, Offenbach-Germany (H. Nier)
2000-2001	Emergency and Trauma Service as Emergency Doctor Nordwest Hospital, Teaching Hospital of the University of Frankfurt-Germany (Prof. H. Bockhorn)
2001	Course in Handsurgery (Prof. J. Böhler) in Vienna, Austria

2001	Course Laparoscopic Plastic Surgery, Ethicon Inc., Hamburg-Germany
2006	Course Gastroenterologic Surgery, Davos, Switzerland (Prof. Saeger)
2006	Course Adult and Child Reanimation, Mainz, Germany (Dr. Schneider)
2007	Canniesburn Practical Course of Flaps in Reconstructive Microsurgery, Glasgow, Scotland (Prof. D. Soutar)
2007	Course Hand Surgery, University Giessen (Prof. Schnettler)

VISITS AND HOSPITATIONS OVERSEAS

1994	Dept. of Surgery, Kilimanjaro Christian Medical Center, Moshi-Tanzania (S. Lyamuya)
1994	Dept. of Plastic Surgery, Frankfurt-Germany (Gottfried Lemperle)
1994, 1996, 1997	Dept. of Plastic- Micro and Hand Surgery, Davies Medical Center, Microsurgical-Institute, San Francisco-USA (Harry Buncke)
1994, 1997	Dept. of Plastic and Reconstructive Surgery, University of California in San Francisco-USA (Stephen Mathes)
1994, 1996, 1997	Dept. of Plastic and Reconstructive Surgery, Stanford University in Palo Alto-USA and Interplast USA (Donald Laub)
1998	Dept. of Burns, Plastic- Reconstructive Surgery, BG-Trauma-Center, University of Heidelberg, Ludwigshafen-Germany (Guenter Germann)

1999	Bernard O'Brien Institute of Microsurgery and Dept. of Plastic Surgery St. Vincent's Hospital, Melbourne, Australia (Wayne Morrison)
1999	Dept. of Pediatric Plastic and Maxillofacial Surgery, University of Melbourne, Royal Children Hospital, Melbourne, Australia (Anthony Holmes)
2006	Dept. of Plastic, Hand and Reconstructive Surgery, Universität Zürich, Schweiz (Dr. Walter Künzi)
2006	Dept. of Plastic, Hand and Reconstructive Surgery, University of Galway, Irland (Dr. John Kelly)
2007	Canniesburn Plastic- and Reconstructive Surgery Unit, University of Glasgow, Scotland (Mr. David Soutar)

DOCTORAL THESIS

1997	„Membranordnung und multiple Chemoresistenz bei Tumorzellen: Untersuchungen an Chinese Hamster Ovary- und Harnblasenkarzinomzellinien mittels Elektronen-Paramagnetischer Resonanzspektroskopie „ ("Membrane Structure and Multidrug Resistance of Tumor Cells: Study of Chinese Hamster Ovary- and Human Bladder Carcinoma Cell Lines by means of Electron-Paramagnetic Resonance Spectroscopy") Awarded with great honors Summa Cum Laude Goethe-University, Center of Surgery, Dept. of Urology, Frankfurt-Germany (Prof. D. Jonas)

RESEARCH ACTIVITIES

1992 - 1997	Surgical Research Lab, Center of Surgery, Dept. of Urology, Goethe-University, Frankfurt-Germany (Prof. D. Jonas) Tumorbiology, Multidrug Resistance, Gene Expression in Cancer Tissue, Cell Culture, Molecular Methods
1997	Surgery Research Lab, University of California in San Francisco-USA (Prof. S. Mathes and Dr. C. Lee) Muscle Flap Ischemia Delay Phenomena-Preperation, Molecularbiological Detection, Gene Expression
1998- 2001	Dept. of Surgery, Nordwest Hospital, Teaching Hospital of the University of Frankfurt-Germany (Prof. H. Bockhorn) and the Surgical Research Lab, Center of Surgery, Goethe-University, Frankfurt-Germany (Prof. D. Jonas) Micrometastasis, Surgical Oncology, Molecular Detection Methods, Cell Culture, Gene Expression, Immunohistochemistry
2001 - 2004	Research Microsurgery Fellowship at the Bernard O'Brien Institute of Microsurgery and Dept. of Reconstructive Surgery St. Vincent's Hospital, University of Melbourne, Melbourne-Australia (Prof. Wayne Morrison) Tissue engineering, Microsurgery, Adipogenesis, Cell Culture, Gene Expression, Immunohistochemistry, Molecular Methods
2005 - 2007	Surgical Research Lab, Center of Surgery, Goethe-University, Frankfurt, Germany (Prof. D. Jonas), Leibniz Research Institute, Magdeburg, Germany (Dr. U. Schröder), Dept. of Anatomy, Magdeburg University, Magdeburg, Germany (Prof. Schwegler) Tumor Marker, Therapeutic Cancer Drugs, Gene Expression, Micrometastasis, Immunohistochemistry, Surgical Oncology, Molecular Detection Methods, Cell Culture

2007- today	Own Research Lab of the University of Tuebingen, Tumor Marker, Therapeutic Cancer Drugs, Gene Expression, Micrometastasis, Immunohistochemistry, Surgical Oncology, Tissue Regeneration, Molecular Detection Methods, Cell Culture

REVIEW ACTIVITY

for peer-review Journals

- Tissue Engineering

PUBLICATIONS

see attached publication list

TEACHING EXPERIENCE

1998-2001	Bedside Teaching in surgery of final year medical students groups Lectures and Examination in surgery of student nurses
2001-2004	Teaching in Surgery of Intern Doctors (junior house officer)
2001-2004	Supervision of Research of PhD-Students
2004 –today	Bedside Teaching in surgery of final year medical students groups Lectures and Examination in surgery

AWARDS & SCHOLARSHIPS

1998	„Summa cum Laude" – Award for MD-Thesis of the Goethe-University Frankfurt, Germany
1998	Carl-Oelemann-Award of the Medical College of Surgeons of the State Hessen, Frankfurt, Germany
2001	Research Award of the German Research Society (DFG) in Bonn, Germany Scholarship for Research Fellowship in Melbourne-Australia
2003	Award of the Surgical Research Society of Australasia, Melbourne, Australia
2005	Best abstract of the German Research Society Meeting 2005, Frankfurt, Germany

GRANTS

1999	Hoffman LaRoche, Basel, Switzerland Research Grant – Tumor Marker in colorectal Cancer
2000	Aventis, Frankfurt Research Grant – Tumor Marker in colorectal Cancer
2001 – 2003	German Research Society Research Grant DO 739/1-1

MEMBERSHIPS

- German College of Surgeons
  - Study Group of Biomaterial, Molecular Diagnostic and Therapy,
  - Surgical Oncology
  - Section of Surgical Research

- European Society for Surgical Research (ESSR)

- Interplast Germany

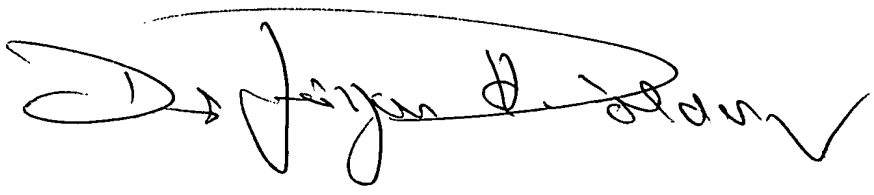
- German Cancer Society

- Paul-Ehrlich-Society (PEG)

- European Tissue Engineering Society (ETES)

- Society of German Reconstructive Surgeons (DGPRAC)

- German Society for Cell- and Tissue Culture (GZG) and German Section of the European Tissue Culture Society (ETCS)



Tübingen, June 20, 2008